# A novel mutation of the *COMP* gene in a Thai family with pseudoachondroplasia

VORASUK SHOTELERSUK and RACHANEEKORN PUNYASHTHITI

Section on Medical Genetics and Metabolism, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand

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**Abstract.** Pseudoachondroplasia (PSACH) is an autosomal dominant disorder characterized by disproportionate short stature and precocious osteoarthritis. Radiographic manifestations include epiphyseal, metaphyseal and vertebral abnormalities. Mutations in the cartilage oligomeric matrix protein (*COMP*) have been identified to cause PSACH. Most of them affect one of the eight calcium-binding domains of *COMP*. We describe a clinically and radiologically typical PSACH 4-year-old girl and her 31-year-old father. A novel mutation, 1345-1347CCC deletion in exon 13, of *COMP* was identified in both patients. The deletion would be expected to result in the loss of the conserved proline at codon 449 from the sixth calcium-binding domain. This result further supports that *COMP* is the only gene, discovered to date, responsible for PSACH across different populations and that the calcium-binding domains are important to the function of the normal *COMP*.

## Introduction

Pseudoachondroplasia (PSACH) is an autosomal dominant disorder characterized by disproportionate short stature, excessive ligamentous laxity, and precocious osteoarthritis with normal face and intelligence (OMIM 177170). Radiographic manifestations include epiphyseal, metaphyseal and vertebral abnormalities (1,2). Tubular bones are short, metaphyses are irregular and widened, epiphyses are small and fragmented, and vertebrae demonstrate flattening and anterior beaking. In 1995, it was demonstrated that mutations in the cartilage oligomeric matrix protein (*COMP*) cause PSACH (3,4). At least 48 mutations have been identified, almost all affecting one of the eight calcium-binding domains. We describe a PSACH Thai girl and her father with a novel mutation in a calcium-binding domain, emphasizing its importance to the normal function of *COMP*.

*Correspondence to*: Dr Vorasuk Shotelersuk, Section on Medical Genetics and Metabolism, Department of Pediatrics, Sor Kor Building 11th floor, King Chulalongkorn Memorial Hospital, Bangkok 10330, Thailand
E-mail: vorasuk.s@chula.ac.th

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## Materials and methods

*Case report.* A female infant was born at term by cesarean section due to cephalopelvic disproportion to a 27-year-old G1P0 Thai mother and a 27-year-old unrelated Thai father. The pregnancy was uncomplicated. Birth weight was 3,000 g and birth length was 50 cm. APGAR scores at 1 and 5 min were 10 and 10, respectively. Physical examination at birth was unremarkable. She had been healthy until she was 18 months old when her parents first noticed that she had short stature, bowed legs and waddling gait. At age 3 years, she could ride a tricycle, speak in sentence, and tell a story. Her father was short but her mother had normal stature. The father completed his bachelor degree in computer. He had complaint of occasional back and knee pains. However, he had never undergone surgery. Besides the 2 individuals, there were no other family members with short stature. History of consanguinity was denied.

Physical examination of the girl at age 4 years revealed height of 83.8 cm (-5 SD), weight 12.3 kg (-2 SD) and OFC 49.5 cm (mean). Arm span was 76.3 cm. The upper to lower trunk ratio was 1.51. Her craniofacial appearance was normal. The lengths of her arms were 12.5 cm, forearms 10 cm, total hands 10 cm and palms 6 cm. The extensions of her shoulder, elbow, hip and knee joints were limited. Flaring and ulnar deviations of her wrists were present. She had genu varum and mild scoliosis (Fig. 1A and B).

Physical examination of her father at age 31 years revealed height of 113 cm, OFC 58 cm, arm span 102 cm, upper to lower trunk ratio 2.05, arms 20.5 cm, forearms 14.5 cm, total hands 12.8 cm, and palms 7.5 cm. His craniofacial appearance was normal. He had mild pectus excavatum and mild scoliosis. The extensions of his shoulder, elbow, hip and knee joints were limited (Fig. 1C and D).

Radiographic findings of the patient revealed short tubular bones, irregular and widened metaphyses, small and fragmented epiphyses, varus deformity of elbows and knees, bullet-shaped vertebral bodies, small odontoid process and slant and irregular acetabuli (Fig. 2A, C, and E). Radiographic manifestations of her father were similar, but much more severe, to his affected daughter (Fig. 2B, D, and F).

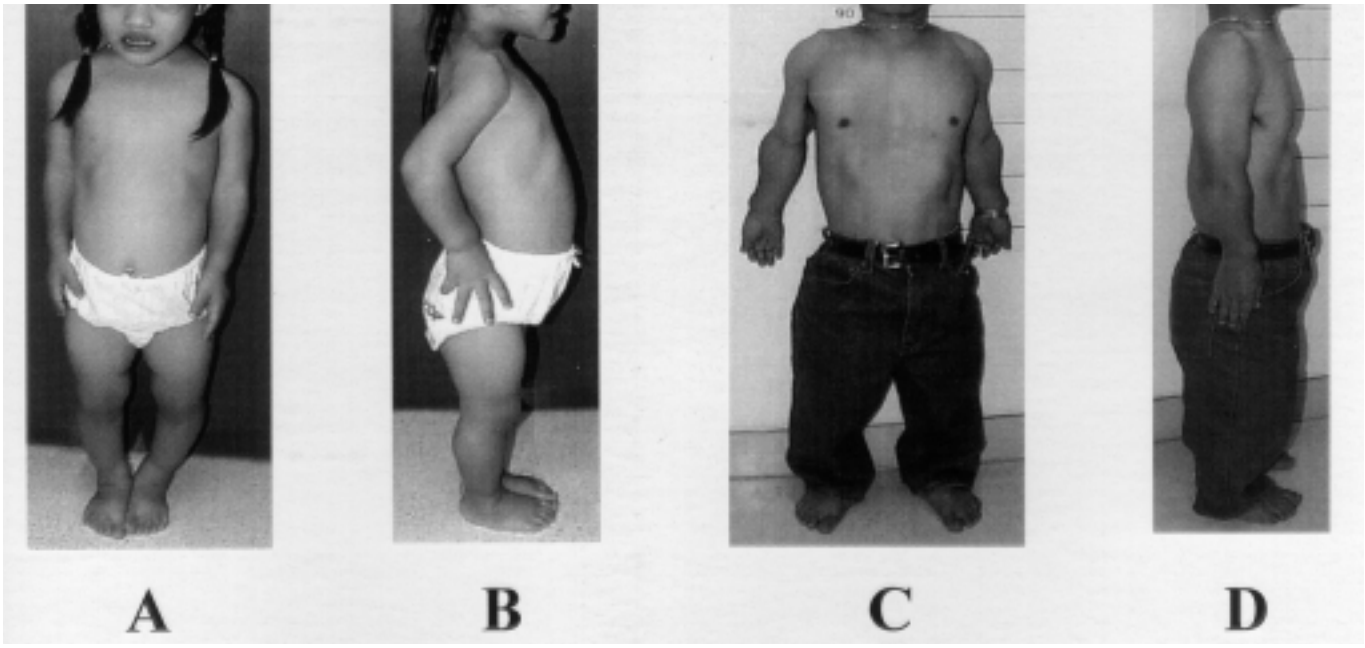


Figure 1. The patient (A and B) and her father (C and D). Total body (A and C): note the normal facial appearance, short limbs, and genu varus. Side (B and D): note the lumbar lordosis and the slight ulnar deviations of wrists.

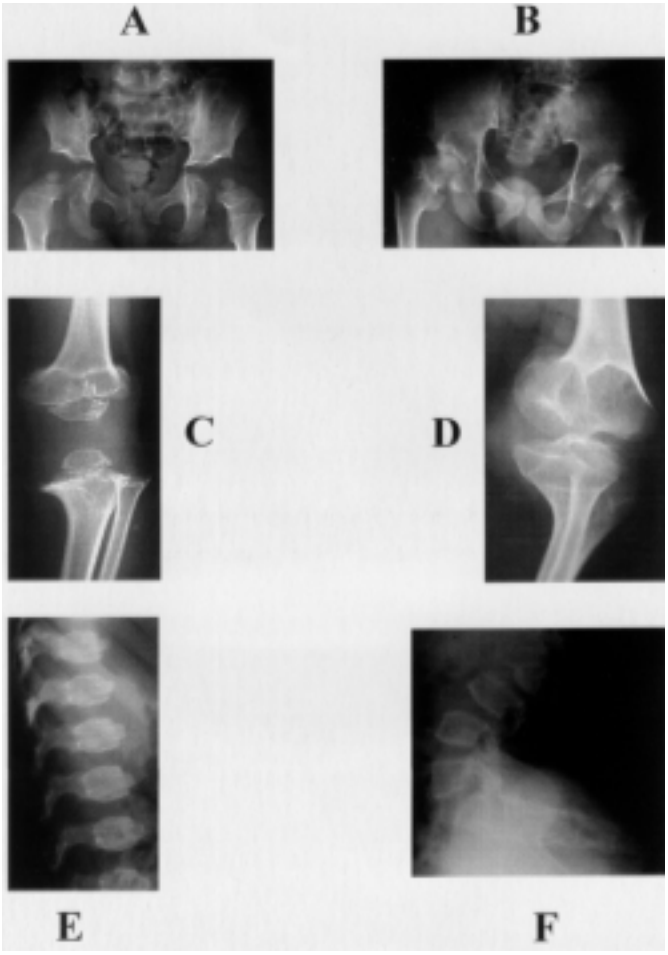


Figure 2. Radiographs of the patient (A, C, and E) and those of her father (B, D, and F). Hips (A and B): note that the acetabuli are irregular, the epiphyses (A) and the femeral heads (B) are small. Left knee (C and D): note the abnormal epiphysis and metaphysis in the child (C). Spine (E and F): note the flattened, bullet-shaped vertebrae (E) and the anterior wedging of L2 and the severe lordosis of the lumbosacral junction (F).

## Results

After informed consent was obtained in accordance with the standards set by local institutional review boards, 3 ml of peripheral blood of the patient and her parents were obtained for DNA isolation by a standard method. Because most identifiable mutations in patients with pseudoachondroplasia are in exon 13 (exon 17B of the thrombospondins) (5) of *COMP*, we designed a nested PCR with the forward primer: 5'-TGGAGAGCTCATTGTCTCTG-3' and the backward primer: 5'-ACCTTGTCTGCATCAAAGTCG-3' for the first round PCR, giving a 385 bp product. For the second round PCR, we used the forward primer: 5'-TCCCACCTATCC ACTCT-3' and the backward primer: 5'-GCCCGCCCACCG TAGAC-3' to amplify a 276 bp product. For both round PCR amplifications, 35 cycles were performed at 94˚C for 30 sec, 58˚C for 30 sec, and 72˚C for 45 sec, followed by an additional extension step at 72˚C for 10 min. The PCR products were electrophoresed on a 2% agarose gel (Promega) and stained with ethidium bromide. The visualized bands were extracted and purified with a kit (Bio 101), and sequenced in both directions by using an automated DNA sequencer (ABI Prism 310 Genetic Analyser, Perkin Elmer).

Direct sequencing analysis of the PCR products revealed that both patients were heterozygous for a deletion of 3 bp, CCC, from nucleotide 1345 to 1347 (Fig. 3). Sequence of the girl's mother was normal.

## Discussion

We describe a clinically and radiologically typical PSACH 4-year-old girl and her 31-year-old father. The girl's birth weight and length were within the normal ranges. The initial features were a short stature and an abnormal gait, appearing in her toddler stage. The father had premature severe osteo-

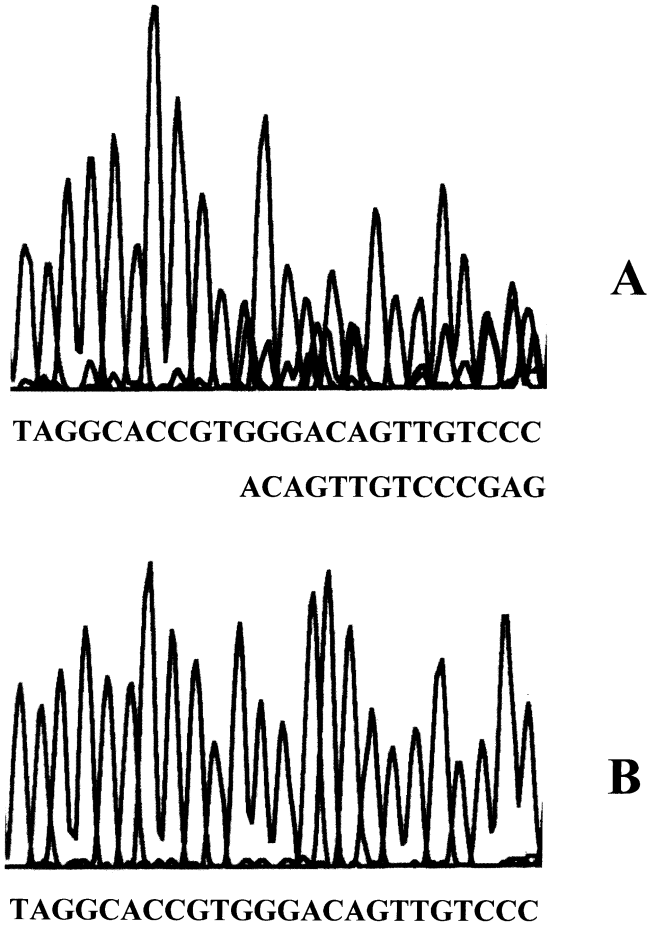


Figure 3. Sequencing from the 3' to 5' direction of the patient (A) and her mother as a control (B). Note the normal sequence can be observed along with the mutant (3 bp deletion) sequence.

arthritis but had not undergone any operations and had no extraskeletal complications, consistent with the typical natural history of patients with PSACH (6). Our patients demonstrated bowing of legs, the most common skeletal complication occurring in 83.8% of patients with PSACH (6). Odontoid hypoplasia present in both patients occurs in approximately half of PSACH individuals (7).

A 1345-1347CCC deletion in exon 13 of *COMP* was identified in both patients. Human *COMP* is a 524-kDa homopentameric glycoprotein expressed prominently in the matrix surrounding chondrocytes (8,9). It is one of the members of the thrombospondin gene family. The monomer contains an amino-terminal domain, four contiguous epidermal growth factor-like repeats, eight contiguous calcium-binding calmodulin-like repeats, and a carboxyl-terminal globular domain (10). At least 48 mutations have been identified in the *COMP* resulting in a single amino acid substitution, a small deletion, or a small insertion. Most known mutations affect residues in the calcium-binding domains with only 4 mutations locating in the carboxyl-terminal domain (3,4,11-22). The 3-bp deletion found in our patients would be expected to result in the loss of the conserved proline at codon 449 from the sixth calcium-binding domain. This proline residue is present in five of the eight calcium-binding domains. A mutation at the same codon, P449T resulting from a 1345C→A mutation, was previously reported to be responsible for PSACH in a family (19). However, no mutations in the other four proline residues have been identified in PSACH individuals. The loss of the residue in our patients would presumably affect the structure of the polypeptide chain. If the mutant and the normal monomer are equally produced, only 1 in 32 pentameric COMP will be completely normal, while others would have at least one arm with a mutation, supporting the hypothesis that the *COMP* mutations act in a dominant negative manner (3,4).

In summary, we describe a PSACH Thai girl and her father with a novel mutation in the sixth calcium-binding domain of *COMP*. This result further supports that *COMP* is the only gene, discovered to date, responsible for PSACH across different populations and that the calcium-binding domains are important to the function of the normal *COMP*.

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